International Journal of Agricultural Science and Research (IJASR)
ISSN(P): 2250-0057; ISSN(E): 2321-0087
Vol. 5, Issue 3, Jun 2015, 293-298
TJPRC Pvt. Ltd.

MOLECULAR CHARACTERIZATION OF BER HYBRIDS AND THEIR PARENTS USING RAPD MARKERS

SURESH KUMAR, S. K. SEHRAWAT & RAJESH LATHAR

Department of Horticulture, CCS Haryana Agricultural University, Hisar, Haryana India

ABSTRACT

Ber, being a hardy and drought tolerant fruit crop, fits well into the marginal ecosystem of semi-arid and arid zones. Twelve ber hybrids and their parents were molecularly characterized using RAPD markers. Of the 35 random primers tested, 26 primers showed amplification of which 24 generated polymorphic bands, while 2 of the primers showed monomorphic bands. Fifteen primers amplified all the ber hybrids and their parents. Five primers (OPE-1, OPE-2 OPE-11, OPE-13 and OPA-18) amplified specific band in ber hybrid or their parent. In total, 165 bands were produced, of which 144 bands were polymorphic, while 21 bands were monomorphic. For the genotypes tested, between 2-24 bands were obtained for each primer with an average of 6.34 bands per primer. The highest number of bands that is 24 were generated by OPE-1. The amplified DNA fragments ranged from 100-800 base pair. With un-weighted group method using arithmetic mean (UPGMA) cluster analysis, twelve ber hybrids and their parents fell into two major clusters.

KEYWORDS: Ber, *Ziziphus mauritiana*, RAPD, Molecular Characterization

INTRODUCTION

Ber or Indian jujube (*Ziziphus mauritiana* Lamk.) is one of the most ancient and common fruit indigenous to India. It belongs to family Rhamnaceae. It grows throughout the tropical, subtropical and arid region (4). It is quite popular due to low cost of cultivation, wide adaptability, ability to withstand drought and good economic returns.

Ber is widely cultivated in Punjab, Haryana, Uttar Pradesh, Rajasthan, Gujarat, Madhya Pradesh, Maharashtra and to some extent in several other states of India. There are more than 125 cultivars grown in India (4). These cultivars have been developed by selection in different regions. Lot of confusion exists in classification or naming of these cuitivars. Apart from morphological characterization, it is desirable to develop alternative methods, which are rapid, reliable and more or less not influenced by environment. Analysis of polymorphism at molecular level can differentiate the genotypes which are non-distinguishable by other tests. Presently more and more scientific work is underway to develop suitable molecular techniques for assisting in plant breeding, genetic engineering and varietal identification. Polymerase chain reaction (PCR) based Random Amplified polymorphic DNA (RAPD) markers have been extensively used in DNA finger printing (1).

MATERIALS AND METHODS

Twelve ber hybrids and their parents were selected from experimental orchard of Department of Horticulture, CCS Haryana Agricultural University, in order to explore the possibility of RAPD markers to detect polymorphism in ber. Genomic DNA was isolated from I to 2 weeks old young, not fully expanded leaves of ber genotypes following CTAB extraction method of Murray and Thompson (2). DNA extracted contained very high amount of RNA and polysaccharides.

DNA sample was treated with RNAse (50 µg/ml) and incubated in water bath at 37ºC for 4 hours to remove RNA contamination from DNA samples. DNA of the genotypes which did not form a clear solution and showed gelatinous appearance was purified by phenol purification. A total of 35 unique 10-base random oligonucleotide primers were used to find out polymorphism among the ber hybrids and their parents. PCR was carried out in 20 µ1 of reaction mixture containing 50 ng of genomic DNA, 1.5 units of Taq DNA polymerse, 2 µl of 10 X Taq DNA polymerase buffer (10 mM Tris-HCI (pH 9.0), 50 mM KCI, 0.1% Triton X-100), 1.5 mM $MgCl_2$, 100 µM of each dNTP's and 0.2 µM of primer. Amplification was carried out in PTC-100 programmable thermal cycler (MJ Research). PCR conditions for RAPD analysis included an initial predenaturation step of 3 minutes at 94ºC and followed by 45 cycles of amplification: denaturation at 94ºC for 1 min, annealing at 40ºC for 1 min, extension at 72ºC for 3 min and final extension was carried out at 72ºC for 15 minutes. Amplified DNA fragments were resolved by submerged horizontal electrophoresis in 1.0 per cent agarose gel and visualized by staining with ethidium bromide. PCR amplification products were viewed by fluorescence under UV light (high UV wavelength 350 nm). Molecular weight of different fragments was determined by using EcoRI-Hind III double digest of λ DNA as standard marker. The gel was photographed using VDS Image Master of Pharmacia Biotech.

The banding patterns from RAPD analysis for each primer were scored by visual observation. The presence of an amplified band in each position was recorded as 1 and absence as 0. Data generated from detection of polymorphic fragments were analyzed by Nei and Li (3) equation.

$$\text{Similarity (F)} = \frac{2Mx}{My + Mz}$$

Dissimilarity = I-F

Where,

Mx = Number of shared fragments between genotypes Y and Z.

My = Number of scored fragments of genotype Y.

Mz = Number of scored fragments of genotype Z.

Based on the pair-wise dissimilarities, cluster analysis was done using unweighed pair group cluster analysis by arithmetic means (UPGMA).

RESULTS AND DISCUSSIONS

Thirty five random decamer primers obtained from operon technologies, USA having 60 per cent or more G+C content were used for RAPD analysis of different ber hybrids and their parents to detect polymorphism. Out of thirty five primers twenty six primers showed amplification (Table 9), fifteen primers amplified all the ber hybrids and their parents. A total of 165 clear and reproducible bands were obtained from 26 primers. For the genotypes tested, between 2-24 bands were obtained for each primer with an average of 6.34 bands per primer. The highest number of bands, that is 24 were generated by OPE-1 (CCCAAGGTCC) followed by OPE-2 (GGTGCGGGAA), which gave 14 bands. The descripton of RAPD primers, which generated polymorphism and number of bands scored at each one is shown in Table 1. A large

number of polymorphic bands were generated by the primers. The polymorphic bands size ranged from 100-800 base pair for the primer OPE-1 and OPE-9. The lowest number of polymorphic bands was generated by primers OPA-5, OPA-11, OPA-17 and OPA-18 which gave one band each. The average per cent polymorphism was 79.84 per cent. The high level of polymorphism obtained is due to the fact that ber possesses a mating system of out crossing, which has resulted in maintenance of high levels of genetic variability in the gene pool, which is reflected in the ber genotypes, selected for study.

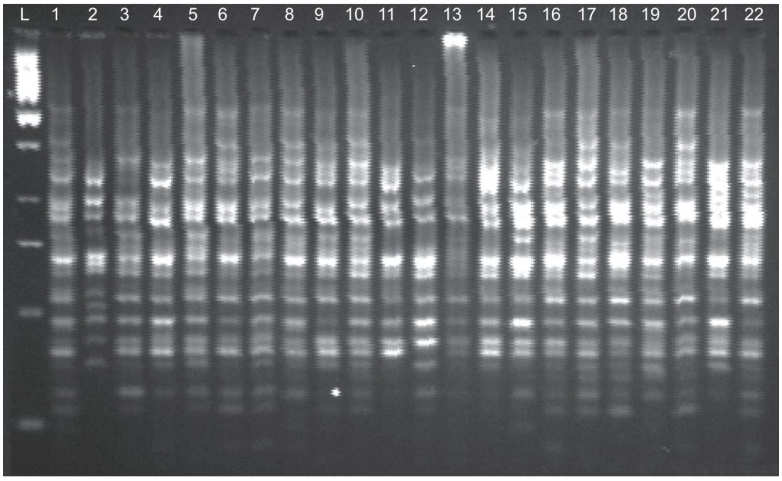

Figure1: Lane L λ DNA Marker, Lane 1-12 Ber Hybrids and Lane 13-22 Parents Showing Polymorphism Generated by OPE-1

Table 1: Random Primers Used for Amplification of Genomic DNA of Ber Hybrids and Their Parents

Total Primers used	35
Primers which showed amplification	26
Primers which are polymorphic	24
Primers which are monomorphic	2
Total number of bands produced	165
Number of polymorphic bands	144
Number of monomorphic bands	21
Average no. of bands per primers	6.34
Percentage of Polymorphism	79.84

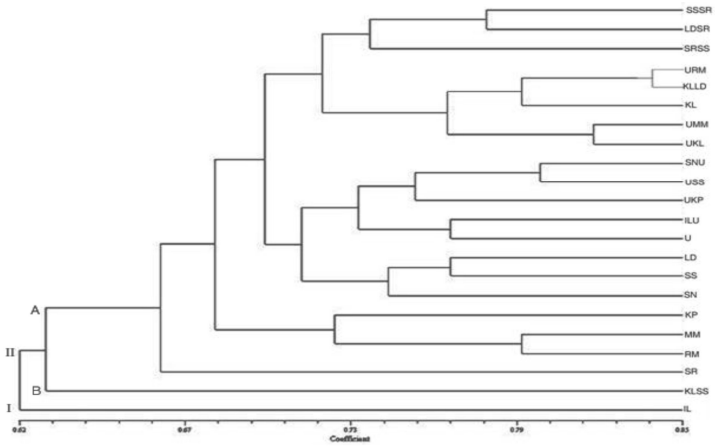

Figure 2: Dendrogram (NTSYS-PC) Showing Genetic Relationships among Ber Hybrids and Their Parents Based on RAPD Markers Analysis

Abbreviations given to Different ber Hybrids and their Parents

Table 2

Hybrids	Female Parent	Male Parent	Abbreviations
Hybrid-1	**Umran**	**Kaithli**	UKL
Hybrid-2	**Umran**	**Safeda selected**	USS
Hybrid-3	**Laddu**	**Safeda Rohtak**	LDSR
Hybrid-4	**Kaithli**	**Safeda Selected**	KLSS
Hybrid-5	**Umran**	**Mundia Murhara**	UMM
Hybrid-6	**Kaithli**	**Laddu**	KLLD
Hybrid-7	**Umran**	**Reshmi**	URM
Hybrid-8	**Illaichi**	**Umran**	ILU
Hybrid-9	**Umran**	**Kathaphal**	UKP
Hybrid-10	**Sandhura Narnaul**	**Umran**	SNU
Hybrid-11	**Safeda Selected**	**Safeda Rohtak**	SSSR
Hybrid-12	**Safeda Rohtak**	**Safeda Selected**	SRSS

Table 3

Parental Genotypes	Abbreviations
Umran	U
Kaithli	KL
Safeda Selected	SS
Laddu	LD
Safeda Rohtak	SR
Mundia Murhara	MM
Reshmi	RM
Illaichi	IL
Kathaphal	KP
Sandhura Narnaul	SN

The UPGMA dendrogram generated from similarity matrix, based on molecular characterization is depicted in Figure2. In the dendrogram, the ber hybrids and their parents were broadly divided into two major clusters at a similarity coefficient of 0.52-0.85. Cluster I consisted of IL (Illaichi) and cluster II comprised of the remaining ber hybrids and their parents and was sub-divided into two sub-cluster, A and B. Sub-cluster A had 20 ber hybrids and their parents which were placed according to their similarity with each other whereas Hybrid KLSS (Kaithli x Safeda Selected) is present alone in sub-cluster B.

It is postulated that in RAPD reaction the comparison of amplified products is determined by a comparison between the potential priming sites in the template DNA rather than by the total number of priming sites available. Therefore, a variation in the amplification patterns and polymorphism is observed for different primers (5). One of the advantages of RAPDs method is that the entire plant genome is targeted for primer annealing, facilitating development of a higher density map.

RAPD markers are mostly dominant and detect variation in both coding and non-coding region of genome and provides an approach to know the exact genetic diversity existing among the genotypes. RAPD analysis is technically simple and provided an approach to characterize different ber genotypes, thus to estimate genetic diversity which will be

useful to breeders in for improvement of ber. Polymorphism can be successfully scored and used for studying genetic variation and diversity.

CONCLUSIONS

No single primer was able to distinguish all the ber hybrids and their parents, however, all the ber hybrids and their parents were distinguishable with the combination of polymorphic bands generated by various primers. The ability to differentiate all the tested hybrids and their parents by RAPD bands suggests that this technique may be practically applied for ber hybrids and their parents identification.

A similarity matrix was generated by NTSYS-PC and the similarity co-efficient ranged from 0.52 to 0.84 depicting a high genetic variability. The highest genetic similarity (0.84) was noticed between Hybrid URM and Hybrid KLLD, whereas Hybrid KLSS and Hybrid USS and IL and MM found to be genetically most diverse (0.52).

With UPGMA cluster analysis based on molecular characterization all the ber hybrids and their parents were distinctly divided into two major clusters. Cluster I consisted of IL (Illaichi) and cluster II comprised of the remaining ber hybrids and their parents.

REFERENCES

1. Luro, F., Laigret, F. and Murie, B. 1995. DNA amplified finger printing. A useful tool for determination of genetic origin and diversity analysis in citrus. *Hort. Science* **30** (5): 1063-1067.
2. Murray, M.G. and Thompson, W.F. 1980. Rapid isolation of high molecular weight plant DNA. *Nucleic Acids Res.:* 4321-4325.
3. Nei, M. and Li., W. 1979. Mathematics model for studying genetic variations in terms of restriction endonucleases. *Proceedings of National Academy of Science,* USA 76: 5269-5273.
4. Pareek, O.P. 2001. *Ber*. International Centre for underutilized crops, Southampton, U.K.
5. Rafalaski, J.A., Tingely, I.V. and Williams, J.G.K. 1991. RAPD markers a new technology for genetic mapping and plant breeding. *Agbiotech, News and Information* **3**: 645-648.